Toxicological Centre
University of Antwerp

The Pigeon Poppy Seed Defence
Analysis of Opiates as Markers of Doping Use in Racing Pigeons

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Case background

In Autumn 2016 several price-winning names in Belgian pigeon racing were accused of using morphine as performance-enhancing drug on their pigeons. One of the accused contacted the University of Antwerp's Toxicological Centre for a second appeal.

To investigate if the poppy seed defence was applicable to this case, an analytical method was developed to detect the 5 most common opiates in avian faeces.

Experimental design

This study was approved by the University of Antwerp's Ethical Committee for Animal Welfare. (LA 1100161)

6 female birds were provided by the accused pigeon fancier. Poppy seeds were purchased from a local supermarket. Wash-out periods were included before and after each experimental block.

Condition A (Days 9-10)
Doping use:
- 172.5 mg poppy seeds
- 1 capsule forced administration
- Collection every 3 hrs for 2 days

Condition B (Days 16-17)
One-time exposure:
- 172.5 mg poppy seeds
- Mixed with food (starvation)
- Collection every 3 hrs for 2 days

Condition C (Days 23-29)
Food contamination:
- 172.5 mg poppy seeds
- Mixed daily with food (excess)
- Collection every 12 hrs for 7 days

Condition D (Days 30-36)
Food contamination:
- 172.5 mg poppy seeds
- Mixed daily with food (starvation)
- Collection every 12 hrs for 7 days

Condition E (Days 37-38)
Doping use (repeat):
- 172.5 mg poppy seeds
- 1 capsule forced administration
- Collection every 3 hrs for 2 days

Sample preparation & analysis

0.5 g faeces + 1.5 mL MeOH + ISTD
- 15 min ultrasonification
- 10 min centrifugation @ 3500 rpm
- Evaporation @ 30 °C

→ Matrix transfer

25 µL β-glucuronidase/arylsulphatase
- 1 mL Na-acetate buffer (1 M; pH 4.5)
- 1 h @ 50 °C

→ Deconjugation

Bond Elut Certify I
- Non-polar C8 – cation exchange mixed-mode
- 2x 3.060 mL DCM:iPA (80:20) + NH_4OH
- Evaporation @ 30 °C

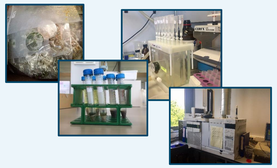
→ Solid Phase Extraction

35 µL BSTFA + 1 % TMCS
- 35 µL ethyl acetate
- 20 min @ 70 °C

→ Derivatisation

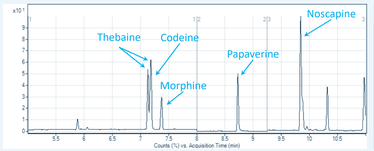
Agilent GC-QQQ 7000B series
- 12 min + post-run
- 150-320 °C @ 20 °C/min
- Electron impact

→ GC-QQQ analysis



Results & Discussion

GC-QQQ analysis



Compound	Pure poppy seeds	Transitions	Calibration range
Thebaine-TMS	12.23 µg/172.5 mg	383.2 → 353.1 / 368.1 / 283.1	4 – 4000 ng/g faeces
Codeine-TMS	8.56 µg/172.5 mg	371.2 → 234.1 / 229.1 / 178.0	4 – 4000 ng/g faeces
Codeine-d6-TMS	-	377.2 → 237.1 / 235.1 / 184.0	@ 100 ng/g faeces
Morphine-2TMS	20.57 µg/172.5 mg	429.2 → 146.0 / 287.1 / 236.2	4 – 4000 ng/g faeces
Morphine-d6-2TMS	-	435.2 → 149.0 / 293.1 / 239.2	@ 100 ng/g faeces
Papaverine	0.09 µg/172.5 mg	338.2 → 322.1 / 307.1 / 278.1	4 – 4000 ng/g faeces
Noscapine	0.22 µg/172.5 mg	220.1 → 205.1 / 118.1 / 147.1	4 – 4000 ng/g faeces

Deuterated internal standards for thebaine, papaverine and noscapine were not available at the time of analysis. Codeine-d6 was used for the quantification of thebaine. Papaverine and noscapine could not be quantified, but were detectable in concentrations as low as 4 ng/g of faeces.

The double peak of thebaine is likely to be due to the presence of stereoisomers and has been reported before by El-Haj et al.

Doping use – Conditions A & E

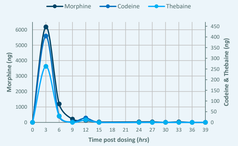


Figure 1. Total amount of opiates found per sampling time after forced administration – data for P3.

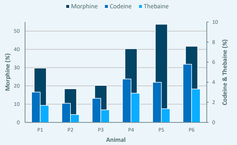


Figure 2. Relative amount of opiates found per animal after forced administration, compared to the theoretically administered dose.

Morphine, codeine and thebaine could be detected in the faeces from the first collection point (3 hrs post dosing), when they would also reach their maximal concentrations. The latest detectable time point for these opiates was around 30 hrs. Suspected enterohepatic recirculation was visible between 12 hrs and 24 hrs post dosing.

Noscapine and papaverine are extensively metabolised to (desmethyl-)meconine and (di-)hydroxypapaverine respectively, and could therefore not be detected.

Total excreted morphine amounts of around 30 % correlate with known human pharmacokinetics.

One-time exposure – Condition B

During most of their races, European pigeons will pass the large poppy fields in France. Any seeds ingested during the race may result in a positive doping test for opiates.

Under experimental conditions the 6 racing pigeons ingested few to none of the poppy seeds, as reflected in the lower concentrations detected (thebaine fell below the detection limit) and the abnormal pharmacokinetic profile compared to controlled administration. The poppy seeds are likely too small for the pigeons to easily pick them up. Visual inspection of the birds' feeder bowls confirmed this suspicion.

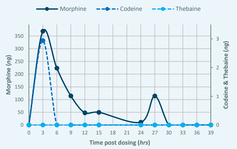


Figure 3. Total amount of opiates found per sampling time after one-time exposure – data for P1.

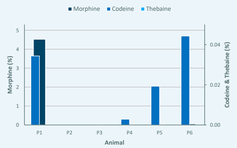


Figure 4. Relative amount of opiates found per animal after one-time exposure, compared to the theoretically administered dose.

Food contamination – Conditions C & D

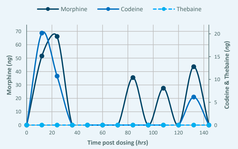


Figure 5. Total amount of opiates found per sampling time after repeated exposure – data for P6.

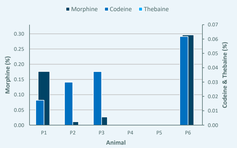


Figure 6. Relative amount of opiates found per animal after repeated exposure, compared to the theoretically administered dose.

The potential for food contamination is high as poppy plants grow in between wheat and barley. Producers of bird food caution that their products may contain poppy seeds, as these are too small to be withheld during the separation of dirt and other unwanted compounds from the bird seeds.

Pigeon fanciers often mix wheatgerm or other oils with the bird seeds to ensure ingestion of even the smallest of seeds and pebbles.

Similar to what was observed during one-time exposure morphine, codeine and thebaine concentrations varied highly between animals and were not comparable to those of conditions A and E.

A day in the life of a research pigeon

Ingesting poppy seeds...

Going about your day...

And being taken care of.

Conclusions

This method can be successfully applied to detect and accurately quantify the opiates present in pigeon faeces.

Morphine, codeine and thebaine were detected in the faeces from 3 hrs up to 30 hrs post dosing. Peak concentrations were reached within 3-6 hrs followed by a steep drop, with signs of enterohepatic circulation after 12-24 hrs. Papaverine and noscapine were both extensively metabolised and present in too low a concentration in the poppy seeds for them to be detected.

Given free choice of intake the birds preferred larger seeds, as had been noted from a visual inspection of the feeders. Starvation did not cause any significant changes in the poppy seed intake.

Case outcome

Both the A and B faeces sample of the accused's allegedly doped pigeons tested positive for the presence of thebaine. The doping laboratory in South-Africa acknowledged these findings indicated the natural origin of the morphine as man-made opiates do not contain this compound.

Investigation of the pigeon food products used (data not shown here) detected the presence of elevated amounts of poppy seeds. Re-analyses by the food manufacturers later confirmed a contamination of the batch in question.

Because of the unusual media attention of this case the Royal Belgian Association of Pigeon Fanciers held a press conference to declare that all charges against had been dropped and to provide further clarifications on the test results.

Further considerations

The short detection window of opiates in the pigeons' faeces (< 48 hrs) makes the use of morphine as doping agent ineffective (the pigeons are released 1 day after leaving the care of the pigeon fancier). Furthermore the samples will not be representative of the conditions during the race as these are obtained 2 days after its finish.

The poppy seeds used were selected for their high opiate content (up to 20 times that of other commercially available brands). On average samples will be even lower in concentration.

Research on the potential beneficial effect of morphine as doping agent in birds is disputed with scientific data lacking.

References

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